



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Chr. Hansen A/S	Examiner:	D.J. Steadman
Serial #:	09/720,096	Group art unit:	1652
Filed:	2 July 1999	Docket:	54337.000009
Title:	Method of preventing bacteriophage infection of bacterial cultures		

DECLARATION BY THOMAS JANZEN

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

I, Thomas Janzen having my residence in DK-1964 Frederiksberg, Ingemannsvej 30, 3. tv,
does state and declare as follows:

1. I am an employee at Chr. Hansen A/S, the assignee of the above patent application, and I hold a position as a research scientist and Section Manager.
2. I am a person skilled in the art to which the above application pertains.
3. I have read and understood the pending claims in that application as well as the office action related thereto dated October 9, 2003, and have the following comments:
4. I have prepared a paper example to demonstrate that the use of *pur⁻* and *thyA* mutants as a bacterial culture as claimed in the above application is just for illustrative purposes and that the illustration should not be interpreted as a limiting feature of the claims.
5. A further example of a bacterial strain having the desired characteristics as recited in the claims is a strain with a partially or completely inactivated DNA polymerase, whereby the DNA polymerase should be partially or completely inactive when the strain is present in milk.

As described on page 12, lines 19-32, of the present specification, such a strain could be constructed by coupling the DNA polymerase gene to an inducible promoter in such a way that the DNA polymerase is expressed under specific culture conditions during strain production, but is not expressed during the growth in milk. The activity of the inducible promoter could as an example be depending on a specific salt concentration or temperature which is present during the production of the strain, but not when the thus produced strain is used in fermentation of milk.

During the propagation of the strain to obtain a culture of said strain, the promoter would be activated enabling the growth of the strain to a required optical density. However, during growth in milk the promoter would be inactive and the DNA polymerase not, or only partly expressed. By this the host chromosomal DNA, as well as potential bacteriophage DNA, would not be replicated in turn leading to phage resistance. The strain would still be metabolically active and thus capable to ferment milk since RNA and proteins (like enzymes) are still synthesized.

13/02/2004 *Thomas Janzen*

It is evident from the above that any bacterial strain listed on page 9, lines 2-7, of the specification would be useful as a parent strain for such a DNA-polymerase mutant.

The characteristics concerning the metabolic activity of such a strain would be comparable to e.g. the disclosed *thyA* mutant, i.e. being metabolically active and thereby enabling an acidification of milk.

6. Thus, the disclosure of specification of the present application is sufficient for me to construct different kinds of bacterial strains which are useful in the claimed method of fermenting milk and which are substantially unaffected by the presence of bacteriophages in said milk.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statement and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United State Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 13/02/2004 Name: Thomas Janzen
month/day/year Thomas Janzen



Curriculum vitae

Thomas Janzen

27.01.2004

Personal History:

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Home Address:

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Educational History:

1984-1991: Christian-Albrechts-University Kiel

Major: Microbiology

Minor: Biochemistry, Zoology

Degree: MSc

Thesis: Plasmid dependent bacteriophage resistance in *Lactococcus lactis* subsp. *cremoris* 11/49 (supervising Professor: Prof. Dr. M. Teuber)

1991-1994: German Dairy Research Institute Kiel

Degree: PhD

Thesis: Plasmid replication in *Streptococcus thermophilus* (supervising Professor: Priv.-Doz. Dr. A. Geis)

Professional Positions:

1994-1997: Chr. Hansen A/S
Research Scientist
Department for Genetics and Microbiology

1997- : Chr. Hansen A/S
Manager Bacteriophage Section
Department of Applied Biotechnology

List of publications

1. **Geis, A., Janzen, T., Teuber, M. and Wirsching, F.** (1992) Mechanism of plasmid-mediated bacteriophage resistance in lactococci. *FEMS Microbiol. Lett.* 94:7-14.
2. **Janzen, T., Kleinschmidt, J., Neve, H., and Geis, A.** (1992) Sequencing and characterization of pST1, a cryptic plasmid from *Streptococcus thermophilus*, *FEMS Microbiol. Lett.* 95:175-180.
3. **Janzen, T.** (1997) Development of dairy cultures with improved phage resistance, Mælkeritidende, Maj 1997.
4. **Hoeier, E. Janzen, T., Henriksen, C.M, Rattray, F., Brockmann, E., and Johansen, E.** (1999) The production, application and action of lactic cheese starter cultures, in: *Technology of cheese making*, ed. by Barry A. Law, Sheffield Academic Press.
5. **Janzen T.** (2002) Forschungsansätze zur Optimierung von Starterkulturen im Hinblick auf ihren Einsatz in der Praxis, Internat. Käseertechnologischen Sonderseminar, Kempten, published in "Deutsche Molkereizeitung".
6. **M.B. Pedersen, P.R. Jensen, T. Janzen, and D. Nilsson** (2002) Bacteriophage resistance of a Δ thyA mutant of *Lactococcus lactis* blocked in DNA replication. *AEM*. Vol. 68, 6: 3010-3023.
7. **Stuer-Lauridsen B., Janzen T., Schnabl J., Johansen E.** (2003) Identification of the host determinant of two prolate-headed phages infecting *Lactococcus lactis*. *Virology* 25, 309(1):10-7.
8. **Hassan A.N., Ipsen R., Janzen T., and Qvist K.B.** (2003) Microstructure and Rheology of Yogurt Made with Cultures Differing Only in their Ability to Produce Exopolysaccharides. *J Dairy Sci.* Vol. 86(5):1632-8.
9. **Madera C., Garcia P., Janzen T., Rodriguez A., and Suarez J.E.** (2003) Characterisation of technologically proficient wild *Lactococcus lactis* strains resistant to phage infection. *Int J Food Microbiol.* 86(3):213-222.
10. **Dupont K., Janzen T., Vogensen F.K, Josephsen J., and Stuer-Lauridsen, B.** (2004) Identification of genes in strains of *Lactococcus lactis* required for phage-binding. Submitted to *AEM*.

Patents

W00/01799: Nilsson, Dan, and Janzen, Thomas. Method of preventing bacteriophage infection of bacterial cultures.
International publication date: 13 January 2000.